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INTRODUCTION

The ease of substitution of azulene in the 1(3)-position under relatively mild conditions by electrophilic reagents has been the subject of many papers concerned with organic synthesis.[1-3] This facility of reaction is probably due to the presence of a zwitterionic structure in the reacting molecule. The blue color of the compound is probably also derived from the zwitterionic structure.[4] The ultraviolet-visible absorption spectrum of azulene in pentane shows the presence of 16 sharp bands with the following wave length maxima and molar absorptivities (in parentheses): 238 (15,000), 270 (50,000), 274 (49,500), 280 (45,000), 296 (3500), 327 (3,000), 338 (3,700), 341 (4,100), 352 (1,100), 540 (210), 560 (260), 582 (322), 605 (290), 633 (320), 660 (152), and 700 (170). In concentrated hydrochloric acid containing 5% by volume of 95% ethanol, azulene has 2 bands at 263 and 353 mμ with molar absorptivities of 22,000 and 13,000, respectively. These spectra are very useful for the characterization of azulene, but in a mixture they might be concealed. For this reason and because of the very low intensity of the visible absorption spectral bands of azulene, analysis for this compound in a mixture is best accomplished with an electrophilic reagent that will give a highly colored cationic chromogen.

Azulene can be detected and determined with 4-dimethylaminobenzaldehyde.[5-7] This reaction has been spectrophotometrically investigated.[8] A blue cationic

chromogen, I, is formed. Two other methods for the detection and determination of azulene and its derivatives have been recently developed.[9] With 2-hydrazinobenzothiazole and an oxidizing agent, azulene compounds give a blue cation, II, the color intensity of which is stable for at least 10 minutes; with 3-methyl-2-benzothiazolone hydrazone azulene compounds give a blue cation, III, the color intensity of which is stable for at least 30 minutes. With the twofold purpose of (a) developing a group of good methods for the detection and determination of azulene compounds under all types of conditions and (b) comparing a large group of electrophilic reagents which could be of possible value in air pollution studies for the analysis of nucleophilic compounds, seven new methods for the determination of azulene are introduced in this paper. The structures for the various chromogens formed are shown in Fig. 1. With indole-3-carboxaldehyde, flavylium perchlorate, xanthydrol, N,N-dimethyl-4-nitrosoaniline, 5-nitroisatin, and 1-anthraquinone diazonium chloride, the dyes IV, V, VI, VII, VIII, and IX were obtained respectively (see Fig. 1 for structures).

The mechanism for the reaction of 4-azobenzenediazonium tetrafluoroborate with azulene is shown in Figure 2.

The advantages and disadvantages of the different reagents will be discussed.

EXPERIMENTAL

Reagents and Equipment. -- 3-Methyl-2-benzothiazolone hydrazone hydrochloride,[10] flavylium perchlorate,[11,12] and 4-azobenzenediazonium tetrafluoroborate[13] were prepared by literature procedures. Indole-3-carboxaldehyde, N,N-dimethyl-4-nitrosoaniline, 5-nitroisatin and the azulene compounds were obtained from the Aldrich Chemical Co. 3-Methyl-2-benzothiazolone hydrazone hydrochloride is also available from the Aldrich Chemical Company.

Xanthydrol and 1-anthraquinonediazonium chloride were obtained from Laboratory Services, Inc., Cincinnati and Dajac Laboratories, respectively. Xanthydrol had to be purified before use. It crystallized from aqueous methanol, m.p. 122-123°C.

A Cary Model 11 recording spectrophotometer with 3-ml 1-cm cells was used in the spectrophotometric procedures.

Definition of Terms. -- Let MW equal the molecular weight of the analyzed compound, C its concentration in moles per liter, ℓ the light path length of the cell in centimeters, A the absorbance, V_c the volume of the cell in milliliters, V_f the final volume of the analyzed solution in milliliters, and V_{ts} the test solution volume in milliliters.

$$\text{Molar absorptivity} = \epsilon = \frac{A}{\ell C} \quad \text{mole}^{-1} \text{ liter cm}^{-1}$$

$$\text{Absorptivity} = K = \frac{\epsilon \times 10^{-3}}{MW} \quad \mu\text{g}^{-1} \text{ ml cm}^{-1}$$

The concentration limit in parts per million refers to the analyzed solution giving an absorbance of 0.1, e.g.,

$$\text{Conc. limit} = \frac{100\ MW}{\epsilon} = \frac{0.1}{K} \text{ ppm}$$

$$\text{Spectral determination limit in micrograms} = \frac{V_f}{V_{ts}} \times \text{Conc. limit}$$

This value represents a readily reproducible, and arbitrary limit of compound giving an absorbance of 0.1. Obviously, a considerable improvement in the determination limit for a compound could be obtained by either decreasing the cell volume or increasing the light path length in the cell. In either case a microbeam condenser or some other change in the optics of the system would be necessary.

Reagent Solutions. -- 4-Azobenzenediazonium tetrafluoroborate, 0.5% solution in 2-methoxyethanol. Stable for at least 4 hours.

Indole-3-carboxaldehyde, 0.25% solution in 2-methoxyethanol.

N,N-Dimethyl-4-nitrosoaniline, 0.5% in 2-methoxyethanol.

5-Nitroisatin chloride. A mixture of 0.13 g. of 5-nitroisatin and 0.14 g. of phosphorus pentachloride in 70 ml of C.P. anhydrous o-dichlorobenzene was refluxed for 5 to 10 minutes, cooled, and then diluted to 100 ml with o-dichlorobenzene. Stable for 4 hours.

Flavylium perchlorate, 0.5% in 2-methoxyethanol.

Xanthydrol, 0.5% in acetic acid: concentrated hydrochloric acid (1:1).

1-Anthraquinonediazonium chloride, 0.25% in 2-methoxyethanol.

4-Azobenzenediazonium tetrafluoroborate procedure A. To 5 ml of the 2-methoxyethanol test solution is added 1 ml of the reagent. The solution is heated 3 minutes on a water bath, cooled to room temperature under tap water, and then diluted to 50 ml with concentrated hydrochloric acid. The absorbance is then read at 620 mμ against the blank. A solution showing a positive reaction has a blue color; the blank is yellow. The color intensity is stable for 1 hour.

Procedure B. To 7 ml of the 2-methoxyethanol test solution is added 1 ml of the reagent. The solution is heated 3 minutes on the water bath, cooled, and diluted to 10 ml with 2-methoxyethanol. The absorbance is then read at 480 mμ against the blank. A solution showing a positive reaction has an orange-red color; the blank is yellow. The color intensity is stable for 30 minutes.

Procedure C. -- The same procedure is used as in B except that the final dilution is with concentrated hydrochloric acid. The absorbance is then read

at 555 mμ against the blank. In the presence of azulene or some of its derivatives a red-violet monocation is formed. The blank is yellow. The data for all the procedures discussed in this paper are given in Table I.

Indole-3-carboxaldehyde Procedure. -- To 5 ml of 2-methoxyethanol test solution is added 3 ml of 0.25% indole-3-carboxaldehyde in 2-methoxyethanol, followed by 2 ml of concentrated hydrochloric acid. After 20 minutes standing the absorbance is read at 570 mμ against the blank.

Flavylium perchlorate Procedure. -- To 5 ml of the 2-methoxyethanol test solution is added 5 ml of the reagent. The mixture is heated 3 minutes on the water bath, cooled, and diluted to 50 ml with acetic acid. The absorbance is then read at 590 mμ.

Xanthydrol Procedure. -- To 5 ml of 2-methoxyethanol test solution is added 10 ml of the reagent. The solution is heated 5 minutes, cooled, and diluted to 50 ml with 2-methoxyethanol. The absorbance is then read at 650 mμ.

1-Anthraquinonediazonium chloride Procedure. -- A mixture of 5 ml of 2-methoxyethanol test solution and 1 ml of reagent is heated 5 minutes on the boiling water bath and cooled. The absorbance of the solution is determined at 540 mμ against a proper blank.

5-Nitroisatin chloride Procedure. -- To 5 ml of the anhydrous o-dichlorobenzene test solution is added 5 ml of the reagent. The mixture is heated on the boiling water bath for 5 minutes, cooled, and then the absorbance is determined at either 605 or 645 mμ against the proper blank.

N,N-Dimethyl-4-nitrosoaniline Procedure. -- To 5 ml of 2-methoxyethanol test solution is added 3 ml of reagent and 2 ml of concentrated hydrochloric acid.

Mixture is heated on the boiling water bath for 5 minutes and allowed to cool. The absorbance is then determined at 622 mμ against the yellow blank.

DISCUSSION

4-Azobenzenediazonium tetrafluoroborate Procedures. -- The effect of varying the conditions in the 3 standard procedures was investigated. Ethanol could be substituted for the 2-methoxyethanol, but the standard procedure had to be modified by allowing the test solution and the reagent to stand for 20 minutes to obtain the maximum color development. To obtain the maximum intensity in the standard procedures the reagent and test solution could be heated from 2 to 25 minutes. The percentage of 4-azobenzenediazonium tetrafluoroborate in the reagent could be varied from 0.05 to at least 1.0% without decreasing the absorbance. Concentrations of the reagent lower than 0.01% gave a drastic decrease in intensity in the procedures.

The effect of acid on the final color and its intensity was very striking when procedure A was modified by dilution with 2-methoxyethanol or a mixture of 2-methoxyethanol and concentrated hydrochloric acid. In a solution containing no hydrochloric acid a wave length maximum of 480 mμ and a molar absorptivity of 35,000 was obtained. The final chromogen was the neutral compound, X. When the final solution contained 5 to 30% by volume of concentrated hydrochloric acid, the monocationic salt, Xa, was formed with a wave length maximum of 557 mμ and a molar absorptivity of 48,000. Finally in procedure A the brilliant blue dicationic salt, Xb, is formed with wave length maximum 620 mμ and molar absorptivity 110,000. This blue compound does not obey Beer's law in the procedure. With lower concentrations of azulene the molar absorptivity increased,

e.g. for 2.56 mμ of azulene per ml of final solution - ε = 92,000, for 0.64 μg - ε = 96,000, and for 0.128 μg - ε = 110,000. A concentration-absorbance curve is necessary for the determination of azulene by this method.

By procedure A, 4,6,8-trimethylazulene gave a blue solution, wave length maximum 623 mμ, and a molar absorptivity of ~90,000 while guaiazulene gave wave length maximum 637 mμ with a molar absorptivity of ~100,000.

The following compounds gave negligible to negative results in the three procedures - pyrrole, phenol, anisole, naphthalene, indole, carbazole, anthracene, pyrene, and benzo[a]pyrene.

Remainder of the Procedures. -- These methods were not as thoroughly investigated as were the 4-azobenzenediazonium tetraflucroborate procedures. However, triplicate determinations were performed in all runs. Beer's law behavior and color stability were determined for all reagents. The data for all the methods are given in Table I.

Comparison of the Procedures. -- The 4-azobenzenediazonium tetrafluoroborate procedure 4 has the highest potential sensitivity in the determination of azulene due primarily to the high color intensity as shown by the molar absorptivity and the absorptivity. The first six procedures have been more thoroughly investigated. It is possible that in the last six procedures higher absorptivities could be obtained by a more thorough examination.

All methods had concentration limits of better than 1 ppm in the final solution. Any particular procedure can be made more sensitive if the dilution factor (e.g. the ratio of the final volume to the test solution volume) can be decreased. This means that dilution factors closer to 1.0 are preferable where

the greatest sensitivity per unit volume of test solution is desirable. Two procedures are given using 3-methyl-2-benzothiazolone hydrazone. The method with a dilution factor of 2 can detect 0.48 ppm of azulene in the test solution, the other method with a dilution factor of 20 can detect 4.8 ppm. In this respect the most sensitive method for azulene is the N,N-dimethyl-4-aminobenzaldehyde procedure which can detect 0.3 ppm of azulene in the test solution. On the other hand the smallest amount of azulene (0.13 μg) can be detected (i.e. giving an absorbance of 0.1) with procedure C of the 4-azobenzenediazonium tetrafluoroborate method.

Beer's law was obeyed in all procedures except in procedure A of the 4-azobenzenediazonium tetrafluoroborate method. The color stabilities and interferences were as shown in Table I. The reproducibility in all procedures over a period of several weeks was within ± 2% of the reported results.

Interferences. -- Other compounds have also been found to react with the reagents described in this paper under somewhat similar conditions. Aniline, the naphthylamines and the anthramines, as well as their alkylated derivatives, react with 4-azobenzenediazonium tetrafluoroborate.[14] α-Naphthol also reacts to give a band near 635 mμ with a molar absorptivity of approximately 6,000. N,N-Dimethyl-p-aminobenzaldehyde reacts readily with pyrroles and indoles to form red to violet dyes.[15] Formaldehyde,[16] aromatic amines,[17] and imino heteroaromatic compounds [17] form brilliant blue chromogens with 3-methyl-2-benzothiazolone hydrazone and an oxidizing agent. 2-Hydrazinobenzothiazole reacts also with formaldehyde to give an intensely blue compound.[18] Flavylium perchlorate gives blue colors on reaction with N,N-dimethylaniline and α-naphthylamine.[19]

Xanthydrol reacts with carbazole to give a blue color.[20,21] 5-Nitroisatin chloride forms brilliantly colored chromogens with N,N-dialkylanilines, diphenylamines, carbazoles, α-naphthol, and indole.[22]

All these reagents have one thing in common; they attack nucleophilic compounds, such as aromatic amines, pyrroles, indoles, phenols, etc. However, azulene and its alkyl derivatives can be readily separated from such compounds by alumina column chromatography if necessary. For the direct analysis of a mixture the N,N-dimethyl-4-aminobenzaldehyde method appears to be the most selective. However, the method of choice for the determination of azulene in a particular complex mixture will depend on the type of interfering compounds present, the sensitivity desired, the amount of sample available, preliminary treatment of the sample, etc.

Many of the chromogens formed in the analytical procedures are new dyes worthy of industrial investigation.

SUMMARY

The colors, wave length maxima, absorptivities, concentration limits, dilution factors, spectral detection limits, Beer's law ranges, color stabilities and interferences of 12 diverse methods for the determination of azulene are compared. Eleven of these methods are new. The molar absorptivities of the different methods range from 17,000 to 110,000. All methods have a concentration limit more sensitive than 1 ppm of azulene; all methods can detect less than 1 μg of azulene. For all procedures the structure of the final chromogen has been deduced.

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TABLE I

DETERMINATION OF AZULENE WITH VARIOUS REAGENTS

Procedure	Color[a]	λ max, mμ	ε x 10-3	Absorptivity μg-1 ml cm-1	Conc. Limit[b,c] pts/millions	Dilution Factor[d]
4-Azobenzenediazonium tetrafluoroborate						
A	B	620	110[e]	0.86	0.11	10.
B	OR	480	35.0	0.27	0.37	1.43
C	RV	557	48.0	0.38	0.27	1.43
N,N-Dimethyl-4-aminobenzaldehyde[8]	B	620	86.0	0.67	0.15	2.0
3-Methyl-2-benzothiazolone hydrazone[9]	B	588	52.6	0.41	0.24	2 or 20.
2-Hydrazinobenzothiazole[9]	B	583	50.0	0.39	0.26	20.
Indole-3-carboxaldehyde	R	570	63.0	0.49	0.20	2.0
Flavylium Perchlorate	G	600	31.0	0.24	0.41	2.0
Xanthydrol	G	650	18.0	0.16	0.61	2.0
1-Anthraquinonediazonium chloride	R	540	38.0	0.29	0.34	1.2
5-Nitroisatin chloride	G	605 645	21.0 21.0	0.16	0.61	2.0
N,N-Dimethyl-4-nitrosoaniline	G	622	17.0	0.13	0.75	2.0

[a] B=blue, O-orange, R=red, V=violet, and G=green.
[b] Calculated for the total volume obtained in procedure.
[c] where absorbance = 0.10 in a 3 ml cell with a light path length of 1 cm
[d] Essentially final volume/test solution volume
[e] Beer's law was not obeyed.
[f] Color reached a maximum intensity at 17 to 30 minutes after the final solution.

TABLE I. cont.

Procedure	Spectral Determination Limit,[b,c] µg	Beer's Law Range[b] µg/ml	Color Stability Min.
4-Azobenzenediazonium tetrafluoroborate			
A	0.33	0.6-20 [e]	120
B	1.1	0.3-10	30
C	0.81	0.2-7	>90
N,N-Dimethyl-4-aminobenzaldehyde[8]	0.45	0.1-3	17-30[b]
3-Methyl-2-benzothiazolone hydrazone[9]	0.72	0.3-32	30
2-Hydrazinobenzothiazole[9]	0.78	0.25-8	10
Indole-3-carboxaldehyde	0.60	0.2-6	>30
Flavylium Perchlorate	1.2	0.4-12	>11
Xanthydrol	1.8	0.6-18	60
1-Anthraquinonediazonium chloride	1.0	0.4-11	>30
5-Nitroisatin chloride	1.8	0.6-16	>60
N,N-Dimethyl-4-nitrosoaniline	2.3	0.8-16	>30

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a B=blue, O=orange, R=red, V=violet, and G=green.
b Calculated for the total volume obtained in procedure.
c where absorbance = 0.10 in a 3 ml cell with a light path length of 1 cm.
d Essentially final volume/test solution volume
e Beer's law not obeyed.
f Color reached a maximum intensity at 17 to 30 minutes after the final dilution.